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Sesquiterpenes and diterpenes from *Ambrosia arborescens*

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ABSTRACT

Six compounds, eudesm-11(13)-en-4β,9β-diol, 15*R*,16-dihydroxy-3-oxo*iso*pimar-9(11)-ene, 15*S*,16-dihydroxy-3-oxo*iso*pimar-9(11)-ene, 1α-hydroxy-7-oxo-*iso*-anhydrooplopanone, 10α-hydroxy-11,13-dihydro-5-*epi*-psilostachyin, and 4β-hydroxypseudoguaian-12,6-olide 4-*O*-β-D-glucopyranoside, together with 12 known sesquiterpenes, were isolated from the leaves of *Ambrosia arborescens*. Structures were elucidated by 1D and 2D NMR spectroscopy including 1D-TOCSY, DQF-COSY, 2D-ROESY, HSQC, and HMBC experiments, as well as by ESI mass spectrometry. The absolute configuration of the 15,16-diol moiety in 15*R*,16-dihydroxy-3-oxoisopimar-9(11)-ene and 15*S*,16-dihydroxy-3-oxoisopimar-9(11)-ene was determined using Snatzke's method. All compounds were evaluated for antiproliferative activity.

1. Introduction

In the course of our studies on South American medicinal plants, we examined studies on the leaves of *Ambrosia arborescens* Mill. (Asteraceae family, tribe Heliantheae, subtribe Ambrosinae), an aromatic plant growing in western South America (Ecuador, Bolivia, Peru) where it is commonly known as "marco" and used traditionally to brush away insects and by shamans to take away ghosts (Correa and Bernal, 1990). All *Ambrosia* species are characterized by a high content of sesquiterpene lactones, which account for antibacterial (Robles et al., 1995), antifungal (Picman, 1984), cytotoxic, and other pharmacological activities (Parkhomenko et al., 2006). Two earlier studies on *A. arborescens* aerial parts reported the isolation of four sesquiterpene lactones, damsin, coronopilin, psilostachyin, and psilostachyin C (Herz et al., 1969; Melchor Lezama and Fuertes Ruiton, 1995).

In the present investigation on *A. arborescens* leaves, we describe the isolation and structural characterization by spectroscopic and spectrometric analyses of six new compounds, four sesquiterpenes, eudesm-11(13)-en-4β,9β-diol (**1**), 1α-hydroxy-7-oxo-*iso*-anhydrooplopanone (**4**), 10α-hydroxy-11,13-dihydro-5-*epi*-psilostachyin (**5**), and 4β-hydroxypseudoguaian-12,6-olide 4-*O*-β-D-glucopyranoside (**6**) and two diterpenes 15*R*,16-dihydroxy-3-oxo*iso*pimar-9(11)-ene (**2**) and 15*S*,16-dihydroxy-3-oxo*iso*pimar-9(11)-ene (**3**), together with 12 known sesquiterpenes (**7**–**18**). All compounds were also evaluated for antiproliferative activity.

2. Results and discussion

The *n*-hexane and chloroform extracts of the leaves of *A. arborescens* were subjected to silica gel column chromatography followed by RP-HPLC, to afford six new terpene derivatives (**1**–**6**) and 12 known sesquiterpenes. The following known compounds were identified by comparison with published spectroscopic data: damsin (**7**) (Herz et al., 1961), psilostachyin (**8**) (Borges del Castillo et al., 1981), volenol (**9**) (Ohmoto et al., 1987; Sun et al., 2004), 12-hydroxy-4(5),11(13)-eudesmadien-15-al (**10**) (Bohlmann et al., 1980), dihydrocoronopilin (**11**) (Geissman and Toribio, 1967), coronopilin (**12**) (Herz et al., 1969; Ata et al., 2007), 4(15)-eudesmene-1β,7α-diol (**13**) (Sun et al., 2004), 13-hydroxy-4-oxo-7(11)-pseudoguaien-12,6-olide (**14**) (Abdel Salam et al., 1984; Jakupovic et al., 1987), 4-(3-oxobutyl)-5-methyl-1-acetic acid-α-methylene-3-cycloheptene (**15**) (Bohlmann and Le Van, 1978; Alberto Marco et al., 1993), damsinic acid (**16**) (Herz et al., 1981), psilostachyin C (**17**) (Borges del Castillo et al., 1981), and 10α-hydroxydamsin (**18**) (Jakupovic et al., 1987).

Compound **1** was assigned molecular formula $C_{15}H_{26}O_2$ by HRESIMS (*m/z* 239.1921 $[M+H]^+$). The ^{13}C NMR and ^{13}C DEPT NMR spectra indicated that **1** contained three CH_3, six CH_2, and two CH carbons, as well as one hydroxymethyne group, and three quaternary carbons. The ^1H and ^{13}C NMR spectra (Table 1) suggested an eudesmane framework with oxygenated functions at C-4 and

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Table 1
^{1}H and ^{13}C NMR data of compounds **1** ($CDCl_3$, 600 MHz) and **4** (CD_3OD, 600 MHz).[a].

	1		**4**	
Position	δ_H	δ_C	δ_H	δ_C
1a	1.75 *br t* (8.2)	38.4	4.85 *br d* (6.0)	71.8
1b	1.43[b]			
2a	2.13 *m*	31.2	2.20 *m*	42.0
2b	1.46[b]		1.80 *ddd* (14.0, 6.0, 5.0)	
3a	1.85 *br t* (13.0)	50.0	3.03 *dt* (9.0, 8.5)	49.0
3b	1.30 *dd* (13.0, 8.3)			
4		71.3	3.18 *m*	55.4
5	1.57[b]	58.8	1.90 *m*	49.0
6a	2.30 *ddd* (14.0, 5.0, 1.8)	35.3	2.37 *dd* (16.0, 3.5)	37.7
6b	2.06 *br dd* (14.0, 6.0)		2.16 *dd* (16.0, 2.0)	
7	2.22 *m*	33.4		203.0
8a	1.78 *m*	32.3		168.6
8b	1.53[b]			
9	3.53 *dd* (12.0, 5.0)	79.6		132.7
10		49.0	1.86 *s*	11.3
11		147.0	1.52 *m*	30.0
12a	4.89 *s*	107.0	0.96 *d* (6.5)	21.2
12b	4.63 *s*			
13	1.52 *s*	30.0	0.80 *d* (6.5)	15.3
14	0.67 *s*	11.7		212.0
15	1.23 *s*	29.7	2.33 *s*	30.0

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments.
[b] Overlapped signal.

Table 2
^{1}H and ^{13}C NMR data of compounds **2** and **3** (CD_3OD, 600 MHz).[a]

	2		**3**	
Position	δ_H	δ_C	δ_H	δ_C
1a	2.09[b]	38.5	2.08[b]	38.5
1b	1.55[b]		1.53[b]	
2a	2.75 *ddd* (15.0, 8.0, 7.5)	35.6	2.73 *ddd* (15.0, 8.0, 7.5)	35.0
2b	2.28 *ddd* (15.0, 9.0, 6.0)		2.28 *ddd* (15.0, 9.0, 6.0)	
3		218.9		219.0
4		48.0		48.1
5	1.56[b]	56.6	1.54[b]	56.3
6a	1.71 *m*	19.3	1.69 *m*	19.5
6b	1.60[b]		1.54[b]	
7a	1.61[b]	24.0	1.60[b]	23.8
7b	1.50[b]		1.57[b]	
8	1.84 *m*	51.0	1.83 *m*	51.0
9		138.0		138.2
10		38.7		38.7
11	5.42 *br s*	129.9	5.48 *br s*	129.8
12a	2.36 *br d* (12.0)	36.2	2.35 *br d* (12.0)	36.4
12b	2.13[b]		2.12[b]	
13		38.6		38.5
14a	1.54[b]	30.8	1.40 *br d* (10.0)	30.8
14b	1.36 *m*		1.30 *t* (10.0, 9.5)	
15	3.34[b]	80.4	3.21 *dd* (9.0, 2.5)	81.3
16a	3.74 *dd* (12.0, 3.0)	63.6	3.63 *dd* (11.5, 2.5)	63.8
16b	3.41 *dd* (12.0, 9.0)		3.45 *dd* (11.5, 8.5)	
17	1.02 *s*	23.0	1.03 *s*	22.8
18	1.11 *s*	22.6	1.10 *s*	22.4
19	1.09 *s*	25.8	1.09 *s*	25.9
20	1.05 *s*	14.6	1.04 *s*	15.0

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments.
[b] Overlapped signal.

C-9 (Xu et al., 2004). A typical doublet of doublets at δ 3.53 (J = 12.0, 5.0 Hz) for H-9α indicated the presence of a hydroxyl group at C-9β. One exocyclic methylene was easily recognizable in the two one proton singlets at δ 4.89 and 4.63. Results obtained from 1D TOCSY and DQF-COSY experiments established the sequences H-1–H-3 and H-5–H-9. All protonated carbons were assigned from the HSQC spectrum. Locations of the hydroxyl groups were confirmed by conducting an HMBC experiment. The signal at δ 3.53 (H-9) correlated with carbon resonances at 11.7 (C-14), 38.4 (C-1), and 49.0 (C-10). The HMBC experiment also indicated connections between δ 1.57 (H-5) and 11.7 (C-14), 33.4 (C-7), 49.0 (C-10), and 79.6 (C-9); δ 1.85 (H-3a) and 29.7 (C-15), 31.2 (C-2), 58.8 (C-5), and 71.3 (C-4); δ 2.22 (H-7) and 32.3 (C-8) and 147.0 (C-11). 2D ROESY correlations between Me-15 and H-9, H-5 and H-7 and H-9 established the relative stereochemistry at C-4, C-5, C-7, and C-9. Thus, **1** was eudesm-11(13)-en-4β,9β-diol.

The molecular formula of **2** ($C_{20}H_{32}O_3$) was determined by HRESIMS ($[M+H]^+$ at m/z 321.2383). The ^{1}H NMR spectrum (Table 2) showed signals for four tertiary methyl groups, a pair of *ddd* centered at δ 2.28 (J = 15.0, 9.0, 6.0 Hz) and 2.75 (J = 15.0, 8.0, 7.5 Hz) for the hydrogens of C-2, and signals for protons at δ 3.34 (overlapped signal), 3.41 (*dd*, J = 12.0, 9.0 Hz), and 3.74 (*dd*, J = 12.0, 3.0 Hz). The ^{1}H NMR spectral data combined with 1D TOCSY and DQF-COSY experiments suggested the sequence C-1–C-2, C-5–C-14, C-11–C-14, and C-15–C-16. The ^{13}C NMR spectrum (Table 2) showed the presence of one keto group, a double bond, and a diol, and was in good agreement with that of a pimarane derivative (Nishiya et al., 1991; Politi et al., 2002). ^{13}C NMR signals were assigned on the basis of an HSQC experiment. Locations of the carbonyl group, the double bond, and the diol were confirmed by analysis of the HMBC spectrum. In fact, the signal at δ 3.74 and 3.41 (H2-16) correlated with carbon resonances at δ 38.6 (C-13) and 80.4 (C-15), the signal at δ 3.34 (H-15) correlated with a signal at δ 63.6 (C-16), leading the location of the diol group at C-15 and C-16; the signals at δ 2.28 and 2.75 (H_2-2) correlated with 38.5 (C-1), 48.0 (C-4), and 218.9 (C-3), and the signal at δ 1.09 (Me-19) correlated with 22.6 (Me-18), 48.0 (C-4), 218.9 (C-3), confirming the location of the keto function at C-3. Finally HMBC correlations between H-12 and C-8, C-9, and C-11 and between H-11 and C-8, C-12, C-13, and C-14 confirmed the C-9/C-11 double bond. 2D ROESY measurements supported the proposed structure and proved the relative stereochemistry at C-5, C-10, and C-13. Thus, the signal of the proton at δ 1.05 (Me-20) correlated with the Me-17 signal, the proton signal at δ 1.84 (H-8) affected the Me-17 and Me-20 signals, while the signal at δ 2.75 (H-2a) weakly influenced Me-17. The absolute configuration of the 15,16-diol moiety of **2** was determined by the circular dichroism (CD) induced after in situ complexation with dimolybdenum tetracetate in DMSO solution (Di Bari et al., 2001). According to a rule proposed by Snatzke (Frelek et al., 1999), the sign of the diagnostic band at about 305 nm correlated with the absolute configuration of the chiral centers in the 1,2-diol moiety. In particular a *R*-monosubstituted glycol gives rise to a negative cotton effect at 305 nm. Thus the negative sign observed in the CD spectrum of **2** allowed us to assign the *R*-configuration to C-15. Consequently, **2** was 15*R*,16-dihydroxy-3-oxo*iso*pimar-9(11)-ene.

Compound **3** was assigned molecular formula $C_{20}H_{32}O_3$ (HRESIMS pseudomolecular positive ion peak at m/z 321.2383). The spectral data (Table 2) of compound **3** indicated its diterpene skeleton. Comparison of its NMR spectra with those of **2** showed that **3** differed from **2** only in the signals due to the 1,2-diol moiety. The absolute configuration of the 15,16-diol moiety of **3** was determined by the same method reported for **2**. The positive sign observed in the CD spectra of **3** led to the assignment of *S*-configuration to C-15. Thus, **3** was identified as 15*S*,16-dihydroxy-3-oxo*iso*pimar-9(11)-ene.

The molecular formula $C_{15}H_{22}O_3$ of **4** was deduced from mass spectral findings ($[M+H]^+$ at m/z 251.1602], ^{13}C NMR and ^{13}C DEPT NMR data, indicating 5 degrees of unsaturation. The ^{13}C NMR and ^{13}C DEPT NMR spectra indicated that **4** contained four methyls,